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TECHNICAL MANUSCRIPT 379

MICROBIOLOGICAL LABORATORY HAZARD
OF BEARDED MEN

Manuel S. Barbeito
Charles T. Mathews
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MARCH 1967

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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Fort Detrick
Frederick, Maryland 21701

TECHNICAL MANUSCRIPT 379

MICROBIOLOGICAL LABORATORY HAZARD OF BEARDED MEN

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Charles T. Mathews

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Agent Control Division
INDUSTRIAL HEALTH AND SAFETY OFFICE

Project 1B622401A072

March 1967

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

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ABSTRACT

An investigation was conducted to evaluate the hypothesis that "a bearded man subjects his family and friends to risk of infection if his beard is contaminated by infectious microorganisms while he is working in a microbiological laboratory." Bearded and unbearded men were tested with Serratia marcescens and Bacillus subtilis var. niger. Contact aerosol transmission from a contaminated beard on a mannequin to a suitable host was evaluated with both Newcastle disease virus and Clostridium botulinum toxin, Type A.

The experiments showed that beards retained microorganisms and toxin despite washing with soap and water. Although washing reduced the amount of virus or toxin, a sufficient amount remained to produce disease upon contact with a suitable host.

I. INTRODUCTION

Indirect contact transmission of disease from the microbiological laboratory to persons outside by means of contaminated clothing has been reported in the instances of Q fever in laundry workers¹ and the veterinarian's wife who may have acquired Q fever by handling the clothing of her husband.²

There may be other cases of similar indirect transmission. However, there are few reports of direct personal contact whereby a healthy microbiological laboratory worker has infected his family or friends outside the laboratory. One paper reported the Q fever infection of a housewife by a tenant in her home; it was concluded that the most reasonable theory was passive carriage of the organism from the laboratory on the clothing, shoes, hands, or hair.³

After many years of absence from the laboratory scene, beards are now being worn by some persons working with pathogenic microorganisms. Beard contamination might result from an evident spill of culture or from an unrecognized microbial aerosol. Previous investigations have shown that common microbiological techniques and accidents generate sufficient microbial aerosol to infect man.⁴ It is assumed that differences in susceptibility may permit infection of a contact even if the bearded carrier remains uninfected. Because the source of laboratory-acquired infection is unknown in 39 to 86% of the cases⁵ it has been our policy that beards are undesirable because they may constitute a risk to close associates.

This hypothesis was tested by four volunteers with 73-day-old beards using noninfective Serratia marcescens and Bacillus subtilis var. niger.

To study transmission of disease by a beard, a full-length natural hair beard on a mannequin was contaminated with Newcastle disease virus and Clostridium botulinum Type A toxin. Chickens and guinea pigs were used as test animals.

II. BACTERIAL EXPERIMENTS WITH BEARDED MEN

A. MATERIALS AND METHODS

1. Bacterial Cultures

Serratia marcescens was grown for 16 hours at 30 C in a modified Bacto tryptose broth medium and diluted immediately before use with physiological saline to a concentration of 1×10^5 organisms per ml.

Bacillus subtilis var. niger was grown for 48 hours at 34 C in a modified N-Z-Amine, Type A, medium and diluted immediately before use with physiological saline to a concentration of 1×10^4 spores per ml.

2. Beard Contamination

One ml of culture was sprayed from a small Chicago atomizer⁶ on the entire beard of each man. In the final experiment, in which one-half the beard was sprayed before shearing off the beard, only 0.5 ml was used. The particles were approximately 3 to 5 microns mass median diameter.

3. Interval between Spraying and Sampling the Beard

Two intervals were used, 30 minutes and 6 hours. The 30-minute interval was selected to represent two work situations: (i) the time necessary for a man to complete a laboratory operation in a zealous attempt to avoid loss of an experimental series despite a known accidental contamination of his beard before he rejoined his associates with an unwashed beard, and (ii) the time required for an immediate shower and change of clothing after an accident that contaminated the beard and before association with fellow employees or family. The 6-hour interval was selected to represent the time between an unrecognized contamination of the beard and family contact with the unwashed beard.

4. Temperature and Humidity Control

An isolated laboratory room served as the test site. During the 30-minute interval between spraying and sampling of the beard, to dry the beard with maximum retention of bacterial viability, the temperature was controlled between 21 and 26 C and the relative humidity was adjusted to 70 to 75%. Preliminary investigation revealed that a relative humidity of about 70% aided organism recovery. Webb⁷ discusses in considerable detail the effect of relative humidity on the decay rate of several microorganisms, including those used in this investigation. Extrapolation from a graph by Webb showed the death rate of cells at 1 hour, 70% relative humidity, and 25 C to be 0.005 for B. subtilis and 0.01 for S. marcescens. During the 6-hour interval temperature and humidity were not controlled; the bearded subjects went about their usual business without, however, doing any microbiological work.

5. Beard Washing

Each man lathered his beard with a soap containing 2% hexachlorophene⁸ and then rinsed it by one of two methods: (i) a splashing method, cupping the hands to catch the water and then splashing the water across the face; or (ii) a shower stream method, placing the face directly under the stream of water from the shower head (Fig. 1). Each method was used by two volunteers. Then the beard was dried with a sterile towel.

6. Recovery of Bacteria from the Beard

Four sampling methods were used on each beard plus a fifth when *S. marcescens* was the test organism. The numbers of organisms recovered by the five methods from each beard were combined into one total, which is shown in Table 1.

1) Each beard was swabbed with six Calgiswabs* (one for each of six different areas) moistened with 1% sodium citrate solution. The Calgiswabs were placed in 4 ml of 1% sodium citrate and the calcium alginate wool⁹ was agitated until dissolved. One-tenth-ml samples were plated in triplicate on corn steep agar plates.¹⁰

2) Then the beard was stroked for 2 minutes with a modified Millipore** filter holder containing a membrane filter connected to the laboratory vacuum. To obtain colonial growth the membrane was aseptically transferred, collecting surface up, to a corn steep agar plate.

3) Six agar impressions were made on each beard with Rodac*** plates containing corn steep agar.¹¹

4) Finally, 250 ml of sterile physiological saline containing 0.1% Naccanol wetting agent was used to rinse each beard and the wash water was collected in a sterile emesis basin. The collected fluid was passed through a membrane filter and the filter placed on a corn steep agar plate.

5) *S. marcescens* - contaminated beards were combed for 1 minute with a sterile aluminum comb fitted with nonabsorbent cotton between the tines. After combing, the cotton was removed aseptically, transferred to a sterile safety blender bowl containing 100 ml of sterile nutrient broth, and mixed for 5 minutes.¹² Five 0.1-ml samples per beard were plated on corn steep agar plates. The five techniques used for bacterial recovery are shown in Figure 2.

* Consolidated Laboratories, Inc., Chicago Heights, Illinois.

** Millipore Filter Corp., Bedford, Mass. 01730.

*** Falcon Plastics Division of B-D Laboratories, Inc., 5500 West 83rd St., Los Angeles, Calif. 90045.



Figure 1. Beard Washing Methods.
A, splashing wash;
B, shower stream wash.

TABLE 1. RECOVERY OF BACTERIA FROM BEARDS

Volunteer	Average Bacterial Colony Count Per Test							
	Serratia marcescens				Bacillus subtilis			
	JFM	JM	TM	MB	JFM	JM	TM	MB
Unwashed Beards								
30 minutes between bacterial spraying and sampling ^{a/}	ND ^{b/}	488	549	858	ND	223	191	320
6 hours between bacterial spraying and sampling	ND	423 ^{c/}	257 ^{c/}	12 ^{c/}	ND	1151 ^{d/}	567 ^{d/}	467 ^{d/}
30 minutes between spraying and shearing of $\frac{1}{2}$ beard ^{e/}								
Chin zone ^{g/}	ND	2303	ND	1217	ND	750	ND	1054
Temple zone ^{g/}	ND	1845	ND	1071	ND	527	ND	1244
Washed Beards								
30 minutes between bacterial spraying and sampling								
Shower stream wash	26	3	ND	ND	3	3	ND	ND
Test positive/ Tests done	3/10	3/10	ND	ND	6/10	3/10	ND	ND
Splashing wash	ND	ND	28	188	ND	ND	5	18
Tests positive/ Test done	ND	ND	7/10	10/10	ND	ND	6/10	9/10
Sheared $\frac{1}{2}$ beard^{e/}								
Shower stream wash								
Chin zone ^{g/}	0	ND	ND	ND	1000	ND	ND	ND
Temple zone ^{g/}	0	ND	ND	ND	0	ND	ND	ND
Splashing wash								
Chin zone ^{g/}	ND	ND	0	ND	ND	ND	(1755) ^{f/}	ND
Temple zone ^{g/}	ND	ND	0	ND	ND	ND	100	ND

a. Four replicate tests per beard. Bacteria were recovered in all four tests.

b. ND = Not done.

c. Two replicate tests per beard.

d. One test per beard.

e. 5×10^4 *S. marcescens* sprayed on one-half of each beard.

f. 3×10^4 *B. subtilis* sprayed on one-half of each beard.

g. This sheared chin zone not washed before shearing.

g. One test per $\frac{1}{2}$ chin or temple. For each man add chin and temple and multiply by two for approximate comparability with other figures.



Figure 2. Techniques for Recovering Microorganisms from Beards.
 Left, modified millipore filter holder, Right, aluminum
 comb fitted with nonabsorbent cotton, Rodac plate.
 Left, Calgiswab, Right, physiological saline rinse.

7. Unwashed Beards

All procedures were as before except that the beards were not washed.

8. Shearing of the Beards

Four bearded zones were designated: right temple, right chin, left temple, left chin. The right side of each beard was sprayed with 0.5 ml *S. marcescens* and allowed to dry for 30 minutes. Two men washed their beards and two did not. The right chin and the right temple zones were separately sheared with a hand scissors and the hair from each zone was collected and separately blended for 2 minutes in a safety blender¹⁸ containing 100 ml sterile nutrient broth. Each of ten replicate 0.1-ml samples of broth from each zone was plated on corn steep agar. The total number of colonies from the ten plates multiplied by 100 was taken as the number of bacteria recovered from each man's half-chin or left or right temple. After the whole face, half bearded and half stubble, had been soaped, washed, dried, rinsed with 70% ethyl alcohol, and air-dried, the process was repeated on the remaining left-side beard, using *B. subtilis* var. niger.

B. RESULTS

Results are summarized in Table 1.

In unwashed beards, when 30 minutes elapsed between spraying of the beard and sampling, more S. marcescens than B. subtilis was recovered. Statistically the difference between the means is significant at about the 10% level. After 6 hours' drying this situation was reversed in accordance with a reported rate of decay in viability of 9.64% per minute for S. marcescens¹³ and a rate of 0.93% per minute for B. subtilis spores.¹³ Statistically the difference between the means is significant at about the 20% level.

In the unsheared beards that were washed after the bacterial spray had dried for 30 minutes, so few bacteria were recovered that statistically there is no significant difference between the means, the two species, and the two washing techniques with the limited number of tests conducted.

Shearing the beard and treating the hair in nutrient broth in a blender increased the number of bacteria recovered from unwashed beards. The importance of this is that the other methods of sampling underestimated the potential infectious dose that a family member might obtain by intimate contact with the unwashed beard. It is evident that family infection is possible if the beard is contaminated by the etiologic agents of such diseases as Q fever, tularemia, Venezuelan equine encephalomyelitis, and West Nile fever, for which the inhaled human infectious dose is about 10 microorganisms or animal infective units.⁴

III. BACTERIAL EXPERIMENTS WITH CLEAN-SHAVEN MEN

A. MATERIALS AND METHODS

Five clean-shaven volunteers tested the persistence of S. marcescens and B. subtilis on the facial skin. The methods of spraying and sampling were the same as those for the bearded men except that the combing method (5) was not used. In all tests there was an interval of 30 minutes between the bacterial spraying and sampling.

B. RESULTS

Results are summarized in Table 2.

Differences between bacterial recovery from the washed attached beard and recovery from the washed face do not seem significant. Thirty minutes after spraying, more bacteria were recovered from the unwashed face than from the unwashed attached beard. But 30 minutes after spraying, more bacteria were recovered from the unwashed hair treated in the blender than from the unwashed face. Data to support this latter observation were obtained by adding the figures for the half-chin and one temple zone of each man and multiplying by two to get an estimate for all the bearded area; e.g., volunteer JM would yield 8,296 and MB 4,576 for S. marcescens, and JM 2,554 and MB 4,596 for B. subtilis, compared with the facial recoveries of 5,074, 1,289, 807, and 1,927 respectively. This suggests that bacteria hold more tenaciously to the beard than to the face. This tentative conclusion is strengthened by noticing that washing the face removes a larger number of bacteria than does washing the attached beard (Table 3).

It seems that, given an equal amount of bacterial contamination, soap and water removes more bacteria from the facial skin than from a beard.

TABLE 2. RECOVERY OF BACTERIA FROM THE FACE OF CLEAN-SHAVEN MEN

Volunteer	Average Bacterial Colony Count Per Test									
	Serratia marcescens ^a					Bacillus subtilis ^b				
	JM	LT	CG	TM	MB	JM	LT	CG	TM	MB
Unwashed Face	5,074	1,289	1,483	469	1,289	807	916	1,744	2,375	1,927
Tests positive/ Tests done	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
Washed Face										
Shower Stream Wash	125	0	0	ND	ND	7	4	1	ND	ND
Tests positive/ Tests done	1/2	0/2	0/2	ND	ND	1/2	2/2	2/2	ND	ND
Splashing Wash	ND	ND	ND	0	0	ND	ND	ND	77	20
Tests positive/ Tests done	ND	ND	ND	0/2	0/2	ND	ND	ND	2/2	1/2

a. Total bacteria sprayed on the face per test was 9×10^4 .b. Total bacteria sprayed on the face per test was 7×10^4 .

TABLE 3. REMOVAL OF BACTERIA BY WASHING

	Average Bacterial Colony Count					
	Face	Beard	Face	Beard	Face	Beard
<u>Serratia marcescens</u>						
Before Washing	5,074	489	469	449	1,289	858
After Washing	125	3	0	28	0	188
<u>Bacillus subtilis</u>						
Before Washing	807	223	2,375	191	1,927	320
After Washing	7	3	77	5	20	18

IV. VIRAL EXPERIMENTS WITH A BEARDED MANNEQUIN

To determine if disease could be transmitted from a contaminated beard to a suitable host by intimate contact, Newcastle disease virus (NDV) of chickens was selected as a test agent.

A. MATERIALS AND METHODS

The chickens* were NDV-free as shown by negative hemagglutination inhibition (HI) activity (beta procedure: constant virus, decreasing serum) run prior to use¹⁴ and by clinical appearance.

With both the washed and the unwashed beards, three tests were run with a virus preparation having a titer of $1 \times 10^{9.7}$ embryo LD₅₀ per ml (ELD₅₀/ml) as calculated by the Reed-Muench method.¹⁵ Another three tests were run with a virus preparation that titered $1 \times 10^{4.7}$.

A mannequin fitted with a sterilized natural hair beard** was placed in a specially equipped plastic exposure chamber within a ventilated gas-tight modular cabinet system.¹⁶ NDV (GB strain) was prepared by harvesting allantoic fluid from previously inoculated White Leghorn eggs. One ml was sprayed on the beard with a small Chicago atomizer. After 30 minutes' drying in the exposure chamber the beard was either washed or not washed,

* Parent stock: female, White Leghorn; male, White Rock or New Hampshire.

** Joseph Aquiar Co., 1034 Stelton Road, Piscataway, N.J. 08854.

depending on the experiment. To test the unwashed beard, the bearded mannequin was passed into a separate contact-exposure section of the gastight cabinet. To test the washed beard, it was removed from the exposure chamber and washed in a separate cabinet with water at 40 C and soap containing 2% hexachlorophene; it was toweled and then replaced in the contact-exposure section. The mannequin was rinsed separately with 70% ethyl alcohol and dried, then transferred to the contact-exposure section for reuse with the washed beard.

Each of three 6-week-old chickens was held with its head alternately nestled in the beard and stroked across one-third of the beard (one chicken on each side and one on the chin) for 5 minutes (Fig. 3). After this contact exposure the chickens were housed individually in ultraviolet-irradiated¹⁷ ventilated cages¹⁸ in another section of the gastight cabinet system. Four control chickens also were placed within the cabinet system; none became infected. To minimize potential transfer of disease by the animal caretaker, a sealed automatic watering device was fabricated for the cages and enough feed was placed in each cage to last for the duration of the experiment.

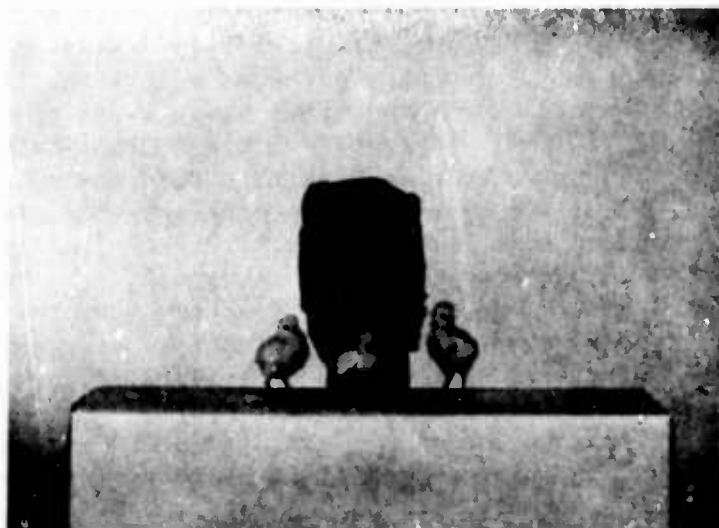


Figure 3. Chickens Exposed to Natural Hair Beard on Mannequin.

1. Recovery of Virus from Lung and Spleen

Four days after exposure to the contaminated beard the chickens were sacrificed. A 1-g sample of spleen and lung tissues from each chicken were ground together in a Ten Broeck mill with 9 ml of sterile tryptose broth containing 5,000 μ g of streptomycin per ml and 10,000 units of penicillin¹⁹ per ml. After centrifugation of the broth at 2,000 rpm for 10 minutes, each of ten 10-day-old embryonated eggs per bird was inoculated in the allantoic cavity with 0.2 ml of the broth supernatant. The eggs were incubated at 37 to 38 C at a relative humidity of 40 to 50%.

All embryos that died within 2 to 6 days were refrigerated overnight, the allantoic fluid was harvested, and a one-tube hemagglutination (HA) test was run.¹⁴ All allantoic fluids with a positive HA test were pooled for each bird (maximum 10 eggs). From this pooled allantoic fluid complete HA tests were run; HI tests were also run with antiserum. Only after HI activity was obtained was the chicken considered positive for contact transmission of the virus from the washed or unwashed beard.

2. Recovery of Virus from Trachea and Brain

One day after exposure to the contaminated beard each separately caged chicken was passed into a special polyvinyl ventilated cabinet. Then each chicken was removed from its cage and a tryptose broth - moistened Swube* was used to swab the larynx and upper trachea for recovery of NDV. The Swube was immersed in 2 ml of tryptose broth containing penicillin and streptomycin and broken apart by vibrating the test tube on a mechanical vibrator. Then 0.1 ml of the broth was injected into the allantoic cavity of 10-day-old embryonated eggs, 10 eggs per bird. Egg handling, incubation, and HA and HI tests were done as described previously. Throat swabs were taken from each bird at 24, 48, and 72 hours after exposure. Birds also were examined for typical symptoms of NDV infection during the holding time. The same titers of virus were used as before.

If chickens from this group died in less than 7 days, the lungs and spleen were ground in tryptose broth; if death occurred between the 7th and 14th day after exposure, 1 g of brain tissue was ground in tryptose broth containing antibiotics. Eggs were inoculated with the supernatant in the same manner as before.

Fourteen days after exposure the surviving chickens were exsanguinated and the NDV HI antibody titer was determined for each blood serum sample by the beta procedure.

* Falcon Plastics Division of B-D Laboratories, Inc., 5500 West 83rd Street, Los Angeles, California 90045.

B. RESULTS

Recovery of virus from lung and spleen is summarized in Table 4. The unpredictable effect of the many variables in this experiment is illustrated by the fact that, among nine chickens, two (numbers 14 and 18) contracted disease by contact with the beard that was washed 30 minutes after it had been sprayed with the low-titered virus, but none of nine chickens (numbers 1 through 9) contracted disease from the high-titered virus. With the unwashed beard the results were consistent in that none of the nine chickens (numbers 46 to 54) was infected by the low-titered contamination and all of the nine chickens (numbers 37 to 45) were infected by the high-titered contamination. But the results with chickens 46 to 54 compared with chickens 14 and 18 reemphasizes the previously mentioned variability of results with chickens 14 and 18.

TABLE 4. RECOVERY OF NEWCASTLE DISEASE VIRUS FROM LUNG AND SPLEEN OF CHICKENS IN CONTACT WITH THE VIRUS-CONTAMINATED BEARDED MANNEQUIN

Beard	Chicken Number	Hemagglutination Inhibition Titer Units ^{a/}
Washed 30 minutes after spraying with		
1 x 10 ^{4.6} ELD ₅₀	14, 18	800
the same	10, 11, 12, 13, 15, 16, 17	0
1 x 10 ^{3.7} ELD ₅₀	1 - 9 incl.	0
Unwashed, sprayed with		
1 x 10 ^{4.6} ELD ₅₀	46-54 incl.	0
1 x 10 ^{3.7} ELD ₅₀	38, 41, 44	800
the same	39, 40, 43	1600
the same	37, 42, 45	3200

a. Reciprocal of dilution.

Recovery of virus from the trachea and brain is summarized in Table 5. Contact by nine chickens with the beard that was washed 30 minutes after it had been sprayed with 1 ml of the high-titered virus, $1 \times 10^{5.7}$ ELD₅₀/ml, resulted in infection of four chickens, numbers 20, 23, 24, 25. None of nine was infected by the low-titered virus in either washed or unwashed beard. All nine chickens numbered 55 through 63 were infected by contact with the unwashed beard sprayed with 1 ml of the high-titered virus.

TABLE 5. RECOVERY OF NEWCASTLE DISEASE VIRUS FROM TRACHEA AND BRAIN OF CHICKENS IN CONTACT WITH THE VIRUS-CONTAMINATED BEARDED MANNEQUIN

Beard	Chicken Number	Hemagglutination Inhibition Titer Units ^a			
		Tracheal Swab	Brain Sample	Lung or Spleen	Blood Serum
		72 Hours After Exposure			
Washed 30 minutes after spraying with					
1 x 10 ^{5.7} ELD ₅₀	19	0			0
the same	20	0			100
the same	21,22	0			0
the same	23	0	1600		
the same	24	0		800	
the same	25	800		800	
the same	26				0
the same	27		0	0	
1 x 10 ^{4.7} ELD ₅₀	64-72 incl.	0			
Unwashed, sprayed with					
1 x 10 ^{5.7} ELD ₅₀	55	800	1600		
the same	58,59,63	800	800		
the same	61	1270	800		
the same	56,57,60	1600	800		
the same	62	3200	800		
1 x 10 ^{4.5} ELD ₅₀	28-36 incl.	0			

a. Reciprocal of dilution.

V. TOXIC EXPERIMENTS WITH A BEARDED MANNEQUIN

To determine if disease could be caused by inhalation and/or ingestion of toxin from a contaminated beard, partially purified Clostridium botulinum Type A toxin was sprayed on the beard and contact was tested with guinea pigs. The guinea pig respiratory LD₅₀ has been reported as 141 mouse intra-peritoneal LD₅₀ (MIPLD₅₀), and the guinea pig oral LD₅₀ as 717 MIPLD₅₀.²⁰

A. MATERIALS AND METHODS

The same test procedures for spraying the material on the beard, washing, handling of the mannequin, animal exposure, and caging were followed as with NDV, except that unventilated, non-UV-irradiated cages were used to house the test animals. The guinea pigs, Hartley strain, weighed between 250 and 300 g each.

The beard was sprayed with 1 ml of partially purified C. botulinum toxin Type A containing 8×10^5 or 8×10^4 MIPLD₅₀/ml.^{21,22} Death within 10 days after exposure was used as the end point to determine toxin transmission from the beard via aerosol and/or oral contact. Sixty guinea pigs were used to make five tests involving three guinea pigs in each test, for each of the two concentrations of toxin that was separately sprayed on the washed beard and on the unwashed beard. During each test the nose and mouth of each of three guinea pigs were nestled and stroked across one-third of the beard for 5 minutes.

B. RESULTS

The results showed no differences between the two test concentrations of 8×10^5 and 8×10^4 MIPLD₅₀/ml of toxin, nor between the washed and unwashed beards. One guinea pig of the 15 exposed in each of the four test groups died within 10 days after exposure.

VI. DISCUSSION AND SUMMARY

Serratia marcescens organisms and spores of Bacillus subtilis var. niger were recovered from washed and unwashed beards, from hair shorn before and after washing, and from washed and unwashed clean-shaven facial skin when microbiological culture recovery techniques were started 30 minutes after the bacteria had been sprayed on the areas. Both species of bacteria were recovered from unwashed beards 6 hours after the bacteria had been sprayed on the beards.

More bacteria could be recovered from clean-shaven facial skin than from the attached beard, and more bacteria were washed off the clean-shaven skin during showering than were washed off the attached beards. Retention of bacteria by the beard was demonstrated by the finding that more bacteria could be recovered from the unwashed beard hair by shearing it off and mixing it in a blender with broth than by recovery techniques used on the attached unwashed beard. This differential retention was not clearly demonstrable in the case of washed beards.

Application of these findings to laboratory situations requires an attempt at quantitation. To obtain culture recovery of bacteria from the washed beard, it was necessary to spray the beard with 1×10^5 S. marcescens organisms or 1×10^4 B. subtilis spores. Fewer would be required for the unwashed beard. Review of the number of S. marcescens organisms recovered by air sampling during simulation of various routine microbiological techniques²³ and recovered immediately after common laboratory accidents, when compared with the dose needed to infect man, shows that (i) most techniques, even when repeated many times, would not contaminate the beard to the 1×10^4 level, and (ii) it is unlikely that the beard would be contaminated with 1×10^4 or 1×10^5 bacteria or viral units without concurrent inhalation of enough organisms to cause illness.

Therefore, infection of family or friends outside the laboratory by an uninfected bearded man would occur only when the bearded man had a recognizable microbiological accident with a persistent highly infectious microorganism or was engaged in a repetitious operation that aerosolized a significant number of organisms and if he himself were protected by vaccination or immunity following clinical or subclinical disease.

A typical repetitious operation would be one on an open bench with Coxiella burnetii, such as grinding in a mortar, using a blender, decanting supernatant, or removing a cotton plug from a shaken culture. In this situation we conclude that (i) a bearded man is a more dangerous carrier than a clean-shaven man because the beard is more resistant to cleansing, and (ii) one working with infectious microorganisms should wash his beard or clean-shaven face before going home.

Results of studies using the bearded mannequin, sprayed with NDV and tested with chickens, or sprayed with botulinum toxin A and tested with guinea pigs, were unexpected because of the large amount of test agent that had to be sprayed on the beard before contact with the washed beard would cause disease in the chickens or guinea pigs. However, the potential for human infection is illustrated by the two chickens that contracted Newcastle disease after contact with one-third of a washed beard sprayed 30 minutes before with $1 \times 10^{4.5}$ ELD₅₀ (31,620 ELD₅₀); in other words, each chicken was in contact with a bearded area sprayed with only 10,540 ELD₅₀ before washing. More impressive is the result with the one guinea pig that obtained a lethal dose of botulinum toxin by contact with a washed beard, one-third of which was sprayed 30 minutes before with 2.66×10^4 MIPLD₅₀; this is equivalent to an estimated 266 human lethal doses. These contaminations are within the range of possible accidental contamination of a beard by a microbial suspension.

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13. ABSTRACT		
<p>An investigation was conducted to evaluate the hypothesis that "a bearded man subjects his family and friends to risk of infection if his beard is contaminated by infectious microorganisms while he is working in a microbiological laboratory." Bearded and unbearded men were tested with <u>Serratia marcescens</u> and <u>Bacillus subtilis</u> var. <u>niger</u>. Contact aerosol transmission from a contaminated beard on a mannequin to a suitable host was evaluated with both Newcastle disease virus and <u>Clostridium botulinum</u> toxin, Type A.</p> <p>The experiments showed that beards retained microorganisms and toxin despite washing with soap and water. Although washing reduced the amount of virus or toxin, a sufficient amount remained to produce disease upon contact with a suitable host.</p>		
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